



TITLE:

Complete Genome Sequences of 13 Human Respiratory Syncytial Virus Subgroup A Strains of Genotypes NA1 and ON1 Isolated in the Philippines

AUTHOR(S):

Malasao, Rungnapa; Furuse, Yuki; Okamoto, Michiko; Dapat, Clyde; Saito, Mayuko; Saito-Obata, Mariko; Tamaki, Raita; Segubre-Mercado, Edelwisa; Lupisan, Socorro; Oshitani, Hitoshi

CITATION:

Malasao, Rungnapa ...[et al.]. Complete Genome Sequences of 13 Human Respiratory Syncytial Virus Subgroup A Strains of Genotypes NA1 and ON1 Isolated in the Philippines. *Genome Announcements* 2018, 6(10): e00151-18.

ISSUE DATE:

2018-03-08

URL:

<http://hdl.handle.net/2433/231087>

RIGHT:

© 2018 Malasao et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



AMERICAN
SOCIETY FOR
MICROBIOLOGY

genomeAnnouncements™

VIRUSES



Complete Genome Sequences of 13 Human Respiratory Syncytial Virus Subgroup A Strains of Genotypes NA1 and ON1 Isolated in the Philippines

Rungnapa Malasao,^{a,b} Yuki Furuse,^{a,c,d,e} Michiko Okamoto,^a Clyde Dapat,^a Mayuko Saito,^a Mariko Saito-Obata,^{a,f} Raita Tamaki,^a Edelwisa Segubre-Mercado,^g Socorro Lupisan,^g Hitoshi Oshitani^a

^aDepartment of Virology, Tohoku University Graduate School of Medicine, Sendai, Japan

^bDepartment of Microbiology, Faculty of Medicine, Chiang Mai University, Chang Mai, Thailand

^cFrontier Research Institute for Interdisciplinary Sciences, Tohoku University, Sendai, Japan

^dInstitute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

^eHakubi Center for Advanced Research, Kyoto University, Kyoto, Japan

^fRITM-Tohoku Collaborating Research Center on Emerging and Reemerging Infectious Diseases, Muntinlupa, Philippines

^gResearch Institute for Tropical Medicine, Muntinlupa, Philippines

ABSTRACT Complete genome sequences of 13 human respiratory syncytial virus strains were determined from samples obtained from children hospitalized in the Philippines between 2012 and 2013 because of acute respiratory infection. We identified amino acid polymorphisms between the NA1 and ON1 genotypes in the P, G, F, and L proteins.

Human respiratory syncytial virus (HRSV) is a major cause of acute lower respiratory tract infection in infants and young children (1). Currently, no vaccine against HRSV is commercially available; the only pharmaceutical option is a monoclonal antibody used for prophylactic treatment of high-risk children (2). Among the HRSV subgroup A viruses, at least 11 genotypes have been identified, including GA1–7, SAA1, NA1, NA2, and ON1 (3, 4). The ON1 genotype, which has a 72-nucleotide duplication in the G gene, was first identified in 2012, and it has replaced its ancestor genotype, NA1, as the predominant genotype in several parts of the world, including the Philippines (5, 6).

We obtained nasopharyngeal swab samples from children hospitalized in the Philippines between 2012 and 2013 because of acute respiratory infection and isolated HRSV strains after culturing the swab samples in HEP-2 cells (5). Thirteen isolates were selected for whole-genome sequencing in the present study. Viral RNA was extracted from culture supernatants using the QIAamp MinElute virus spin kit (Qiagen, Hilden, Germany), and cDNA was transcribed using the SuperScript III reverse transcriptase and random primers (Thermo Fisher Scientific, Waltham, MA). Whole-genome sequences were obtained from 18 overlapping PCR fragments, produced using HRSV-specific primers (7–9). The PCR products were purified using the illustra ExoProStar digestion kit (GE Healthcare, Little Chalfont, UK). Sanger sequencing was subsequently performed using the BigDye Terminator version 3.1 cycle sequencing kit and the 3730 DNA analyzer instrument (Thermo Fisher Scientific, Waltham, MA). Informed consent was obtained from the guardians of all participants. The study protocol was approved by the Ethics Committee of the Tohoku University Graduate School of Medicine, Japan, and the Institutional Review Board of the Research Institute for Tropical Medicine, Philippines.

Among the 13 sequenced isolates, 6 were identified as genotype NA1 and 7 as genotype ON1, both of which belong to HRSV subgroup A. The complete genome

Received 5 February 2018 Accepted 13 February 2018 Published 8 March 2018

Citation Malasao R, Furuse Y, Okamoto M, Dapat C, Saito M, Saito-Obata M, Tamaki R, Segubre-Mercado E, Lupisan S, Oshitani H. 2018. Complete genome sequences of 13 human respiratory syncytial virus subgroup A strains of genotypes NA1 and ON1 isolated in the Philippines. *Genome Announc* 6:e00151-18. <https://doi.org/10.1128/genomeA.00151-18>.

Copyright © 2018 Malasao et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Hitoshi Oshitani, oshitanih@med.tohoku.ac.jp.

sequences of the NA1 and ON1 strains were 15,204 and 15,277 nucleotides long, respectively. As previously reported, the ON1 strains had a 72-nucleotide duplication in the second hypervariable region of the G gene (3). Moreover, we identified a single nucleotide insertion in the ON1 genome located in the untranslated region between the SH and G genes. Comparison of the NA1 and ON1 genomes revealed nonsynonymous substitutions in the genes encoding the P protein (position 92), G protein (positions 126, 130, 232, 237, 253, and 314), F protein (position 104), and L protein (positions 598 and 835).

The reason the ON1 strain has replaced the previously circulating NA1 strain and has become a globally predominant genotype remains unclear (6). The complete genome sequences of the recently circulating strains reported in this study may provide some insights for understanding the genetic diversity and evolution of HRSV.

Accession number(s). The complete genome sequences of the 13 HRSV strains in this study have been deposited at GenBank under the accession numbers [KY654506](#) to [KY654518](#).

ACKNOWLEDGMENTS

We thank the RITM-Tohoku Collaborating Research Team on Emerging and Re-emerging Infectious Diseases.

This work was supported by the Science and Technology Research Partnership for Sustainable Development from the Japan International Cooperation Agency and the Japan Agency for Medical Research and Development (AMED); the Japan Initiative for Global Research Network on Infectious Diseases from the Ministry of Education, Culture, Sport, Science and Technology in Japan and AMED; and KAKENHI (grant JP16H02642) from the Japan Society for the Promotion of Science. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

- Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, Polack FP, Balsells E, Acacio S, Aguayo C, Alassani I, Ali A, Antonio M, Awasthi S, Awori JO, Azziz-Baumgartner E, Baggett HC, Baillie VL, Balmaseda A, Barahona A, Basnet S, Bassat Q, Basualdo W, Bigogo G, Bont L, Breiman RF, Brooks WA, Broor S, Bruce N, Bruden D, Buchy P, Campbell S, Carosone-Link P, Chadha M, Chipeta J, Chou M, Clara W, Cohen C, de Cuellar E, Dang D-A, Dash-Yandag B, Deloria-Knoll M, Dherani M, Eap T, Ebruke BE, Echavarria M, de Freitas Lázaro Emediato CC, Fasce RA, Feikin DR, Feng L, Gentile A, Gordon A, Goswami D, Goyet S, Groome M, Halasa N, Hirve S, Homaira N, Howie SRC, Jara J, Jroundi I, Kartasasmita CB, Khuri-Bulos N, Kotloff KL, Krishnan A, Libster R, Lopez O, Lucero MG, Lucion F, Lupisan SP, Marcone DN, McCracken JP, Mejia M, Moisi JC, Montgomery JM, Moore DP, Moraleda C, Moyes J, Munywoki P, Mutyara K, Nicol MP, Nokes DJ, Nymadawa P, da Costa Oliveira MT, Oshitani H, Pandey N, Paranhos-Baccalà G, Phillips LN, Picot VS, Rahman M, Rakoto-Andrianarivelo M, Rasmussen ZA, Rath BA, Robinson A, Romero C, Russomando G, Salimi V, Sawatwong P, Scheltema N, Schweiger B, Scott JAG, Seidenberg P, Shen K, Singleton R, Sotomayor V, Strand TA, Sutanto A, Sylla M, Tapia MD, Thamthitawat S, Thomas ED, Tokarz R, Turner C, Venter M, Waicharoen S, Wang J, Watthanaworawit W, Yoshida L-M, Yu H, Zar HJ, Campbell H, Nair H; RSV Global Epidemiology Network. 2017. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet* 390:946–958. [https://doi.org/10.1016/S0140-6736\(17\)30938-8](https://doi.org/10.1016/S0140-6736(17)30938-8).
- The Impact-RSV Study Group. 1998. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 102:531–537. <https://doi.org/10.1542/peds.102.3.531>.
- Eshaghi AR, Duvvuri VR, Lai R, Nadarajah JT, Li A, Patel SN, Low DE, Gubbay JB. 2012. Genetic variability of human respiratory syncytial virus A strains circulating in Ontario: a novel genotype with a 72 nucleotide G gene duplication. *PLoS One* 7:e32807. <https://doi.org/10.1371/journal.pone.0032807>.
- Shobugawa Y, Saito R, Sano Y, Zaraket H, Suzuki Y, Kumaki A, Daput I, Oguma T, Yamaguchi M, Suzuki H. 2009. Emerging genotypes of human respiratory syncytial virus subgroup A among patients in Japan. *J Clin Microbiol* 47:2475–2482. <https://doi.org/10.1128/JCM.00115-09>.
- Malasao R, Okamoto M, Chaimongkol N, Imamura T, Tohma K, Daput I, Daput C, Suzuki A, Saito M, Saito M, Tamaki R, Pedrera-Rico GAG, Aniceto R, Quicho RFN, Segubre-Mercado E, Lupisan S, Oshitani H. 2015. Molecular characterization of human respiratory syncytial virus in the Philippines, 2012–2013. *PLoS One* 10. <https://doi.org/10.1371/journal.pone.0142192>.
- Duvvuri VR, Granados A, Rosenfeld P, Bahl J, Eshaghi A, Gubbay JB. 2015. Genetic diversity and evolutionary insights of respiratory syncytial virus A ON1 genotype: global and local transmission dynamics. *Sci Rep* 5. <https://doi.org/10.1038/srep14268>.
- Kumaria R, Iyer L, Hibberd ML, Simões EAF, Sugrue RJ. 2011. Whole genome characterization of non-tissue culture adapted HRSV strains in severely infected children. *Virol J* 8:372. <https://doi.org/10.1186/1743-422X-8-372>.
- Rebuffo-Scheer C, Bose M, He J, Khaja S, Ulatowski M, Beck ET, Fan J, Kumar S, Nelson MI, Henrickson KJ. 2011. Whole genome sequencing and evolutionary analysis of human respiratory syncytial virus A and B from Milwaukee, WI 1998–2010. *PLoS One* 6. <https://doi.org/10.1371/journal.pone.0025468>.
- Tan L, Lemey P, Houspie L, Viveen MC, Jansen NJG, van Loon AM, Wiertz E, van Bleek GM, Martin DP, Coenjaerts FE. 2012. Genetic variability among complete human respiratory syncytial virus subgroup A genomes: bridging molecular evolutionary dynamics and epidemiology. *PLoS One* 7. <https://doi.org/10.1371/journal.pone.0051439>.